

IN THE CLAIMS

1. (original) A method comprising:
- a) reacting two or more samples, each sample comprising one or more reactive analytes, with a different labeling reagent of a set of labeling reagents to thereby produce two or more differentially labeled samples each comprising one or more labeled analytes wherein the different labeling reagents of the set each comprise the formula:



or a salt thereof, wherein;

- i) RG is a reactive group that is a nucleophile or an electrophile and that is capable of reacting with one or more of the reactive analytes of the sample;
  - ii) RP is a reporter moiety that comprises a fixed charge or that is ionizable, wherein the gross mass of each reporter is different for each reagent of the set;
  - iii) LK is a linker moiety that links the reactive group and the reporter group, wherein the mass of the linker compensates for the difference in gross mass between the reporters for the different labeling reagents of the set such that the aggregate gross mass of the reporter and linker combination is the same for each reagent of the set;
  - iv) X is a bond between an atom of the reporter and an atom of the linker;
  - v) Y is a bond between an atom of the linker and an atom of the reactive group, wherein, once the labeling reagent is reacted with the reactive analyte, bond Y links the linker to the analyte; and
  - vi) bonds X and Y fragment in at least a portion of the labeled analytes when subjected to dissociative energy levels; and
- b) mixing two or more of the differentially labeled samples, or a portion thereof, and optionally one or more calibration standards to thereby produce a sample mixture;

wherein RP:

- i) has a gross mass of less than 250 daltons; and/or
- ii) does not substantially sub-fragment under conditions of dissociative energy applied to cause fragmentation of at least a portion of both bonds X and Y of a labeled analyte in a mass spectrometer; and/or
- iii) is not a polymer or is not a biological polymer.

2. (original) A method comprising:

- a) reacting two or more samples, each sample comprising one or more reactive analytes, with a different labeling reagent of a set of labeling reagents to thereby produce two or more differentially labeled samples each comprising one or more labeled analytes wherein the different labeling reagents of the set each comprise the formula:



or a salt thereof, wherein;

- i) RG is a reactive group that is a nucleophile or an electrophile and that is capable of reacting with one or more of the reactive analytes of the sample;
- ii) RP is a reporter moiety that comprises a fixed charge or that is ionizable, wherein the gross mass of each reporter is different for each reagent of the set;
- iii) LK is a linker moiety that links the reactive group and the reporter group, wherein the mass of the linker compensates for the difference in gross mass between the reporters for the different labeling reagents of the set such that the aggregate gross mass of the reporter and linker combination is the same for each reagent of the set;
- iv) X is a bond between an atom of the reporter and an atom of the linker;
- v) Y is a bond between an atom of the linker and an atom of the reactive group, wherein, once the labeling reagent is reacted with the reactive analyte, bond Y links the linker to the analyte; and

- vi) bonds X and Y fragment in at least a portion of the labeled analytes when subjected to dissociative energy levels; and
  - b) mixing two or more of the differentially labeled samples, or a portion thereof, and optionally one or more calibration standards to thereby produce a sample mixture;
- wherein the linker LK undergoes neutral loss under conditions of applied dissociative energy.

3. (original) A method comprising:

- a) reacting two or more samples, each sample comprising one or more reactive analytes, with a different labeling reagent of a set of labeling reagents to thereby produce two or more differentially labeled samples each comprising one or more labeled analytes wherein the different labeling reagents of the set each comprise the formula:



or a salt thereof, wherein;

- i) RG is a reactive group that is a nucleophile or an electrophile and that is capable of reacting with one or more of the reactive analytes of the sample;
- ii) RP is a reporter moiety that comprises a fixed charge or that is ionizable, wherein the gross mass of each reporter is different for each reagent of the set;
- iii) LK is a linker moiety that links the reactive group and the reporter group, wherein the mass of the linker compensates for the difference in gross mass between the reporters for the different labeling reagents of the set such that the aggregate gross mass of the reporter and linker combination is the same for each reagent of the set;
- iv) X is a bond between an atom of the reporter and an atom of the linker;

- v) Y is a bond between an atom of the linker and an atom of the reactive group, wherein, once the labeling reagent is reacted with the reactive analyte, bond Y links the linker to the analyte; and
- vi) bonds X and Y fragment in at least a portion of the labeled analytes when subjected to dissociative energy levels; and
- b) mixing two or more of the differentially labeled samples, or a portion thereof, and optionally one or more calibration standards to thereby produce a sample mixture;

wherein, under conditions of dissociative energy applied in a mass spectrometer, the fragmentation of one of bonds X or Y results in the fragmentation of the other of bonds X or Y.

4. (original) A method comprising:

- a) reacting two or more samples, each sample comprising one or more reactive analytes, with a different labeling reagent of a set of labeling reagents to thereby produce two or more differentially labeled samples each comprising one or more labeled analytes wherein the different labeling reagents of the set each comprise the formula:



or a salt thereof, wherein;

- i) RG is a reactive group that is a nucleophile or an electrophile and that is capable of reacting with one or more of the reactive analytes of the sample;
- ii) RP is a reporter moiety that comprises a fixed charge or that is ionizable, wherein the gross mass of each reporter is different for each reagent of the set;
- iii) LK is a linker moiety that links the reactive group and the reporter group, wherein the mass of the linker compensates for the difference in gross mass between the reporters for the different labeling reagents of

the set such that the aggregate gross mass of the reporter and linker combination is the same for each reagent of the set;

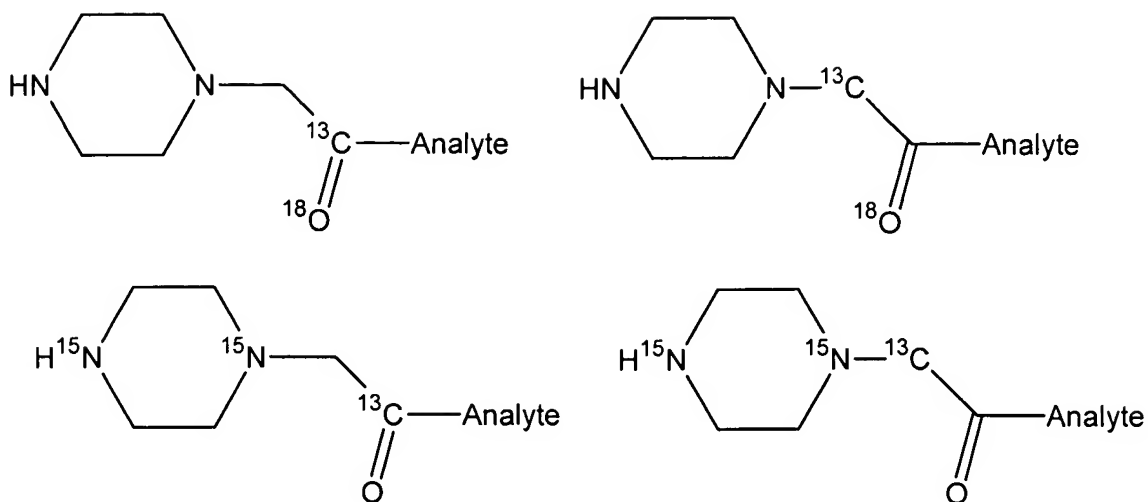
- iv) X is a bond between an atom of the reporter and an atom of the linker;
  - v) Y is a bond between an atom of the linker and an atom of the reactive group, wherein, once the labeling reagent is reacted with the reactive analyte, bond Y links the linker to the analyte; and
  - vi) bonds X and Y fragment in at least a portion of the labeled analytes when subjected to dissociative energy levels; and
- b) mixing two or more of the differentially labeled samples, or a portion thereof, and optionally one or more calibration standards to thereby produce a sample mixture;

wherein:

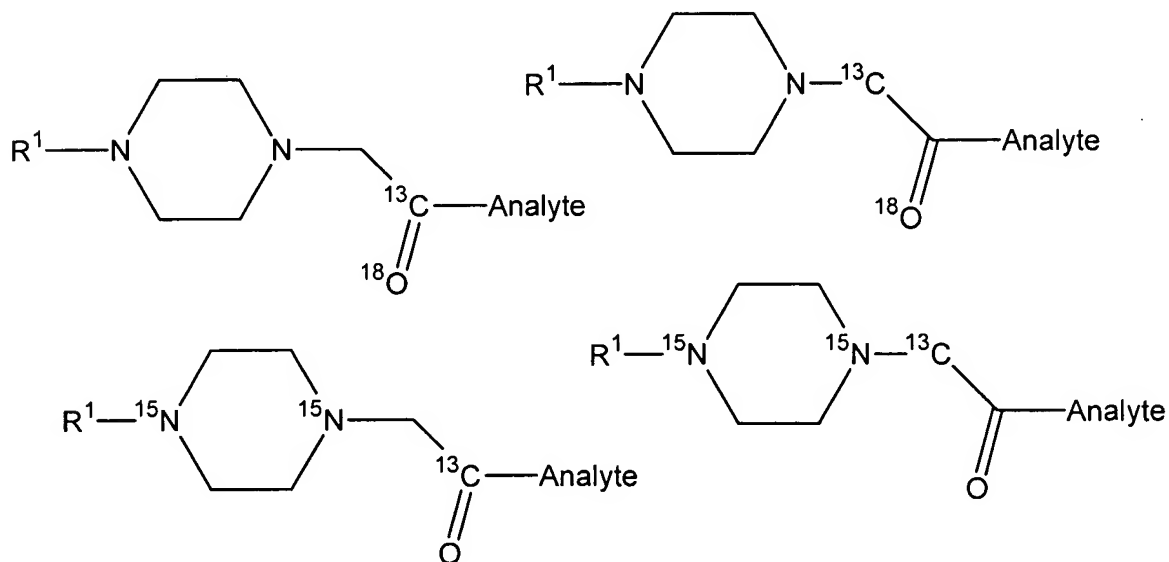
- i) under conditions of dissociative energy applied in a mass spectrometer, bond X is less prone to fragmentation as compared with bond Y; and/or
- ii) under conditions of dissociative energy applied in a mass spectrometer, bond X is less prone to fragmentation as compared with the peptide bond of a Z-pro amino acid dimer or Z-asp amino acid dimer, wherein Z is any natural amino acid, pro is proline and asp is aspartic acid.

5. – 42. (cancelled)

43. (original) The method of claim 40, wherein the sample mixture comprises one or more isobarically labeled analytes of the formula:

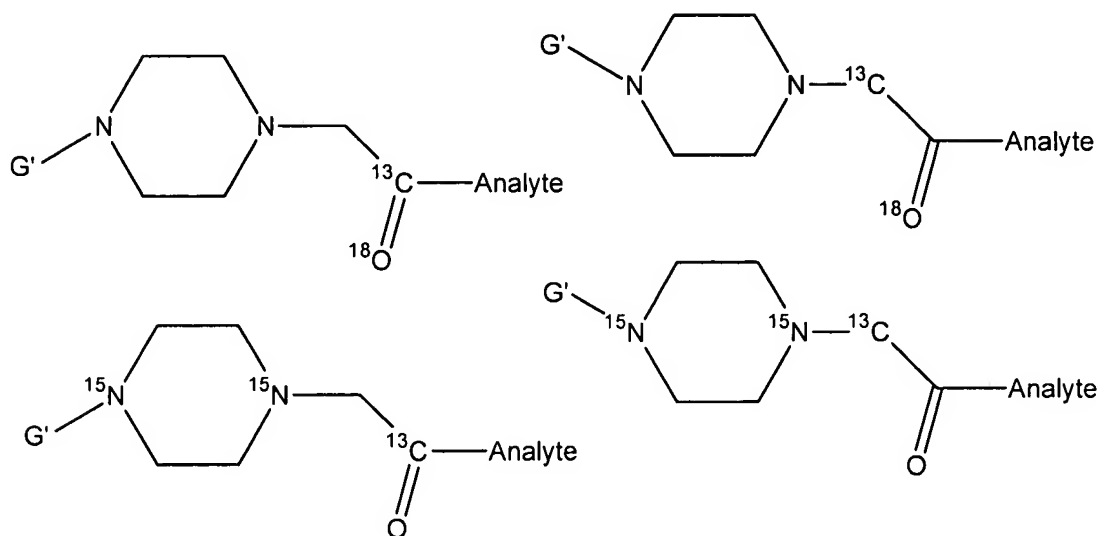


44. (original) The method of claim 40, wherein the sample mixture comprises one or more isobarically labeled analytes of the formula:



wherein each  $R^1$  is the same or different and is an alkyl group comprising one to eight carbon atoms which may optionally contain a heteroatom or a substituted or unsubstituted aryl group wherein the carbon atoms of the alkyl and aryl groups independently comprise linked hydrogen, deuterium and/or fluorine atoms.

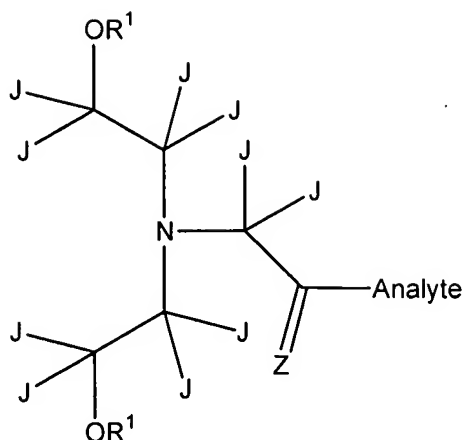
45. (original) The method of claim 40, wherein the sample mixture comprises one or more isobarically labeled analytes of the formula:



wherein:

- G' is an amino alkyl, hydroxy alkyl or thio alkyl group comprising one to eight carbon atoms which may optionally contain a heteroatom or a substituted or unsubstituted aryl group wherein the carbon atoms of the alkyl and aryl groups independently comprise linked hydrogen, deuterium and/or fluorine atoms;
- each carbon of the heterocyclic ring has the formula CJ<sub>2</sub>, wherein each J is the same or different and is selected from the group consisting of: H, deuterium (D), R<sup>1</sup>, OR<sup>1</sup>, SR<sup>1</sup>, NHR<sup>1</sup>, N(R<sup>1</sup>)<sub>2</sub>, fluorine, chlorine, bromine and iodine; and
- each R<sup>1</sup> is the same or different and is an alkyl group comprising one to eight carbon atoms which may optionally contain a heteroatom or a substituted or unsubstituted aryl group wherein the carbon atoms of the alkyl and aryl groups independently comprise linked hydrogen, deuterium and/or fluorine atoms.

46. (original) The method of claim 38, wherein the isobarically labeled analytes in the sample mixture each comprise the formula:



wherein:

- a) Z is O, S, NH or NR<sup>1</sup>;
- b) each J is the same or different and is selected from the group consisting of: H, deuterium (D), R<sup>1</sup>, OR<sup>1</sup>, SR<sup>1</sup>, NHR<sup>1</sup>, N(R<sup>1</sup>)<sub>2</sub>, fluorine, chlorine, bromine and iodine;
- c) each R<sup>1</sup> is the same or different and is an alkyl group comprising one to eight carbon atoms which may optionally contain a heteroatom or a substituted or unsubstituted aryl group wherein the carbon atoms of the alkyl and aryl groups independently comprise linked hydrogen, deuterium and/or fluorine atoms.

47. – 91. (canceled)

92. (original) A mixture comprising at least two labeled analytes, wherein each of the two labeled analytes originates from a different sample combined to form the mixture and each comprises the formula:



or a salt thereof, wherein;

- a) RP is a reporter moiety that comprises a fixed charge or that is ionizable, wherein the gross mass of each reporter is different for each sample;
- b) LK is a linker moiety that links the analyte and the reporter group, wherein the mass of the linker compensates for the difference in gross mass between the

different reporters such that the aggregate gross mass of the reporter and linker combination is the same for each labeled analyte;

- c) X is a bond between an atom of the reporter and an atom of the linker;
- d) Y is a bond between an atom of the linker and an atom of the analyte; and
- e) bonds X and Y fragment in at least a portion of the labeled analytes when subjected to dissociative energy levels; and

wherein RP:

- i) has a gross mass of less than 250 daltons; and/or
- ii) does not substantially sub-fragment under conditions of dissociative energy applied to cause fragmentation of at least a portion of both bonds X and Y of a labeled analyte in a mass spectrometer; and/or
- iii) is not a polymer or is not a biological polymer.

93. (original) A mixture comprising at least two labeled analytes, wherein each of the two labeled analytes originates from a different sample combined to form the mixture and each comprises the formula:

RP-X-LK-Y-Analyte

or a salt thereof, wherein;

- a) RP is a reporter moiety that comprises a fixed charge or that is ionizable, wherein the gross mass of each reporter is different for each sample;
- b) LK is a linker moiety that links the analyte and the reporter group, wherein the mass of the linker compensates for the difference in gross mass between the different reporters such that the aggregate gross mass of the reporter and linker combination is the same for each labeled analyte;
- c) X is a bond between an atom of the reporter and an atom of the linker;
- d) Y is a bond between an atom of the linker and an atom of the analyte; and
- e) bonds X and Y fragment in at least a portion of the labeled analytes when subjected to dissociative energy levels; and

wherein the linker LK undergoes neutral loss under conditions of applied dissociative energy.

94. (original) A mixture comprising at least two labeled analytes, wherein each of the two labeled analytes originates from a different sample combined to form the mixture and each comprises the formula:



or a salt thereof, wherein;

- a) RP is a reporter moiety that comprises a fixed charge or that is ionizable, wherein the gross mass of each reporter is different for each sample;
- b) LK is a linker moiety that links the analyte and the reporter group, wherein the mass of the linker compensates for the difference in gross mass between the different reporters such that the aggregate gross mass of the reporter and linker combination is the same for each labeled analyte;
- c) X is a bond between an atom of the reporter and an atom of the linker;
- d) Y is a bond between an atom of the linker and an atom of the analyte; and
- e) bonds X and Y fragment in at least a portion of the labeled analytes when subjected to dissociative energy levels; and

wherein, under conditions of dissociative energy applied in a mass spectrometer, the fragmentation of one of bonds X or Y results in the fragmentation of the other of bonds X or Y.

95. (original) A mixture comprising at least two labeled analytes, wherein each of the two labeled analytes originates from a different sample combined to form the mixture and each comprises the formula:



or a salt thereof, wherein;

- a) RP is a reporter moiety that comprises a fixed charge or that is ionizable, wherein the gross mass of each reporter is different for each sample;
- b) LK is a linker moiety that links the analyte and the reporter group, wherein the mass of the linker compensates for the difference in gross mass between the

different reporters such that the aggregate gross mass of the reporter and linker combination is the same for each labeled analyte;

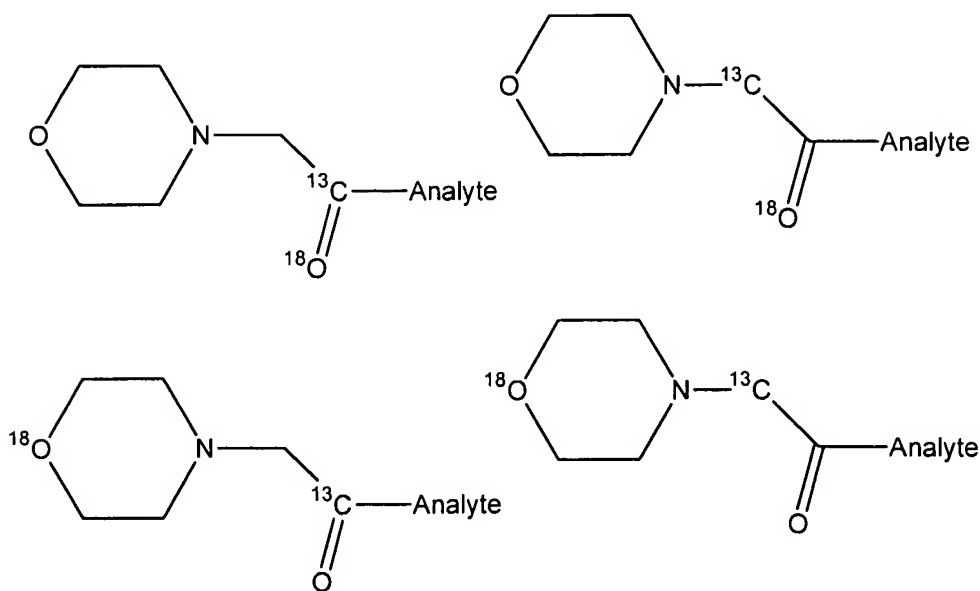
- c) X is a bond between an atom of the reporter and an atom of the linker;
- d) Y is a bond between an atom of the linker and an atom of the analyte; and
- e) bonds X and Y fragment in at least a portion of the labeled analytes when subjected to dissociative energy levels; and

wherein:

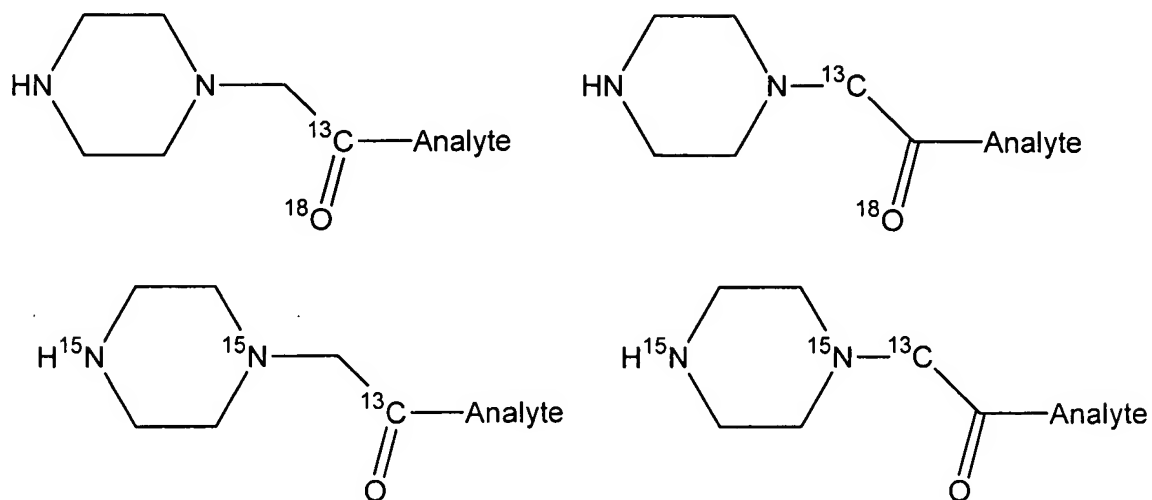
- i) under conditions of dissociative energy applied in a mass spectrometer, bond X is less prone to fragmentation as compared with bond Y; and/or
- ii) under conditions of dissociative energy applied in a mass spectrometer, bond X is less prone to fragmentation as compared with the peptide bond of a Z-pro amino acid dimer or Z-asp amino acid dimer, wherein Z is any natural amino acid, pro is proline and asp is aspartic acid.

96. – 105. (canceled)

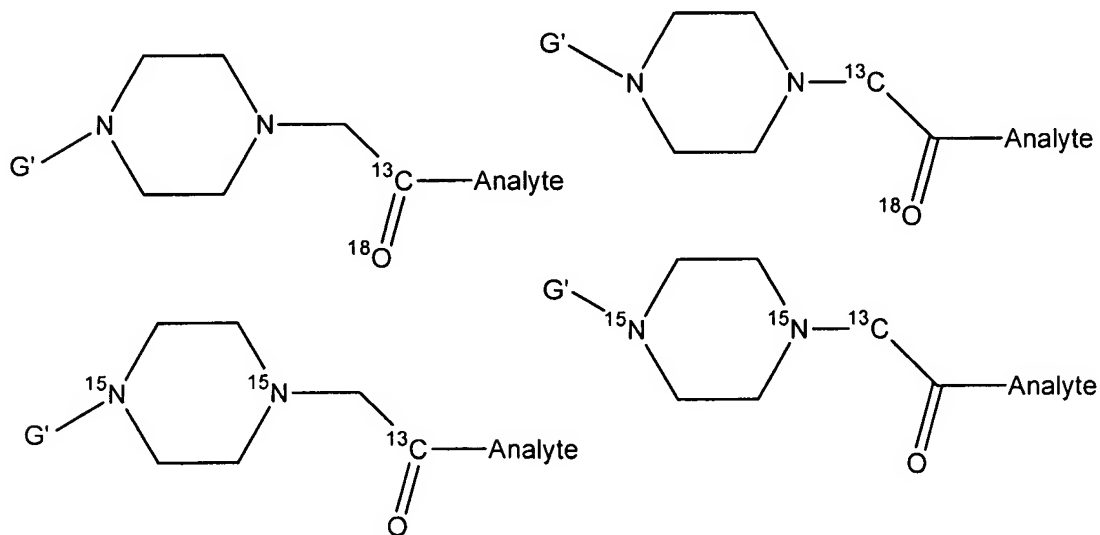
106. (original) The mixture of claim 105, wherein the mixture comprises one or more isobarically labeled analytes of the formula:



107. (original) The mixture of claim 105, wherein the mixture comprises one or more isobarically labeled analytes of the formula:



108. (original) The mixture of claim 105, wherein the mixture comprises one or more isobarically labeled analytes of the formula:



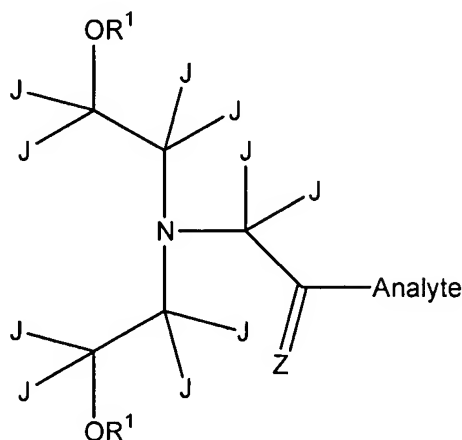
wherein:

- a) G' is an amino alkyl, hydroxy alkyl or thio alkyl group comprising one to eight carbon atoms which may optionally contain a heteroatom or a substituted or unsubstituted aryl group wherein the carbon atoms of the alkyl

and aryl groups independently comprise linked hydrogen, deuterium and/or fluorine atoms;

- b) each carbon of the heterocyclic ring has the formula  $CJ_2$ , wherein each J is the same or different and is selected from the group consisting of: H, deuterium (D),  $R^1$ ,  $OR^1$ ,  $SR^1$ ,  $NHR^1$ ,  $N(R^1)_2$ , fluorine, chlorine, bromine and iodine; and
- c) each  $R^1$  is the same or different and is an alkyl group comprising one to eight carbon atoms which may optionally contain a heteroatom or a substituted or unsubstituted aryl group wherein the carbon atoms of the alkyl and aryl groups independently comprise linked hydrogen, deuterium and/or fluorine atoms.

109. (original) The mixture of claim 103, wherein the mixture comprises one or more isobarically labeled analytes of the formula:



wherein:

- a) Z is O, S, NH or  $NR^1$ ;
- b) each J is the same or different and is selected from the group consisting of: H, deuterium (D),  $R^1$ ,  $OR^1$ ,  $SR^1$ ,  $NHR^1$ ,  $N(R^1)_2$ , fluorine, chlorine, bromine and iodine;
- c) each  $R^1$  is the same or different and is an alkyl group comprising one to eight carbon atoms which may optionally contain a heteroatom or a substituted or unsubstituted aryl group wherein the carbon atoms of the alkyl and aryl

groups independently comprise linked hydrogen, deuterium and/or fluorine atoms.

110. (canceled)